In vitro and in vivo evaluation of \textsuperscript{11}C-SD5024, a novel PET radioligand for human brain imaging of cannabinoid CB\textsubscript{1} receptors

Tetsuya Tsujikawa \textsuperscript{a}, Sami S. Zoghbi \textsuperscript{a}, Jinsoo Hong \textsuperscript{a}, Sean R. Donohue \textsuperscript{a}, Kimberly J. Jenko \textsuperscript{a}, Robert L. Gladding \textsuperscript{a}, Christer Halldin \textsuperscript{b}, Victor W. Pike \textsuperscript{a}, Robert B. Innis \textsuperscript{a}, Masahiro Fujita \textsuperscript{a,\textast}}

\textsuperscript{a} Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA
\textsuperscript{b} Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, Stockholm, Sweden

\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

We recently developed a novel cannabinoid subtype-1 (CB\textsubscript{1}) receptor radioligand \textsuperscript{11}C-SD5024 for brain imaging. This study aimed to evaluate \textsuperscript{11}C-SD5024 both in vitro and in vivo and compare it with the other CB\textsubscript{1} receptor ligands previously used in humans, i.e., \textsuperscript{11}C-MePPEP, \textsuperscript{11}C-OMAR, \textsuperscript{18}F-MK-9470, and \textsuperscript{18}F-FMPEP-d\textsubscript{2}. In vitro experiments were performed to measure dissociation constant ($K_d$) in the human brain and to measure the lipophilicity of the five CB\textsubscript{1} receptor ligands listed above. In vivo specific binding in monkeys was measured by comparing total distribution volume ($V_T$) at baseline and after full receptor blockade. The kinetics of \textsuperscript{11}C-SD5024 in humans were evaluated in seven healthy subjects with compartmental modeling. SD5024 showed $K_d = 0.47$ nM, which was at an intermediate level among the five CB\textsubscript{1} receptor ligands. Lipophilicity ($LogD_{7.4}$) was 3.79, which is appropriate for brain imaging. Monkey scans showed high proportion of specific binding: ~80% of $V_T$. In humans, \textsuperscript{11}C-SD5024 showed peak brain uptake of 1.5–3 standardized uptake value, which was slightly higher than that of \textsuperscript{11}C-OMAR and \textsuperscript{18}F-MK-9470. One-compartment model showed good fitting, consistent with the vast majority of brain uptake being specific binding found in the monkey. Regional $V_T$ values were consistent with known distribution of CB\textsubscript{1} receptors, $V_T$ calculated from 80 and 120 min of scan data was strongly correlated ($R^2 = 0.97$), indicating that 80 min provided adequate information for quantitation and that the influence of radiometabolites was low. Intersubject variability for $V_T$ of \textsuperscript{11}C-SD5024 was 22%, which was low among the five radioligands and indicated precise measurement. In conclusion, \textsuperscript{11}C-SD5024 has appropriate affinity and lipophilicity, high specific binding, moderate brain uptake, and provides good precision to measure the binding. The results suggest that \textsuperscript{11}C-SD5024 is slightly better than or equivalent to \textsuperscript{11}C-OMAR and that both are suitable for clinical studies, especially those that involve two scans in one day.

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\textsuperscript{\ast} Corresponding author at: Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH) and by high peak brain uptake of ~6 standardized uptake value (SUV), a high percentage (85%) of specific binding in the monkey brain, small intersubject variability of total distribution volume ($V_T$) (26%), and a moderate level of retest variability (15%) (Terry et al., 2010). \textsuperscript{11}C-labeled ligands have some advantages over \textsuperscript{18}F-labeled ones because...
the shorter half-life allows more than one synthesis per day using the same hot-cell and the lower radiation-absorbed doses allow more PET scans in each subject. On the other hand, the shorter half-life can make precise quantification difficult if radioligand kinetics are slow or if the concentrations in the brain and plasma are low.

Currently, no clearly good $^{11}$C-labeled PET ligand is available to image the CB$_1$ receptor. Despite the high density of the CB$_1$ receptor, $^{11}$C-OMAR shows peak brain uptake of only 1.5–2 SUV (Wong et al., 2010), which may make accurate quantification difficult. Although $^{11}$C-MePPEP shows high peak brain uptake of 3–4 SUV, washout from brain is too slow for precise quantification possibly due to its high affinity. In addition, intersubject variability for $V_t$ of $^{11}$C-MePPEP is greater than 50% indicating poor precision of the measurement (Terry et al., 2009).

We recently developed a novel CB$_1$ receptor ligand labeled with $^{11}$C from a 3,4-dipropylpyrazoline structural class, namely $^{11}$C-SD5024, [cyano-$^{11}$C](-)-(4-chlorophenyl)-N-[[4-(cyanophenyl)sulfonyl]-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (Donohue et al., 2008). The purposes of this study were two folds, first, to evaluate both in vitro and in vivo the ability of $^{11}$C-SD5024 to quantify CB$_1$ receptors, and second, to evaluate the utility of $^{11}$C-SD5024 relative to other published ligands, particularly the $^{11}$C-labeled ones. For these purposes, we measured in vitro affinity in human brain tissue and lipophilicity of all of the five ligands (SD5024, OMAR, MK-9470, MePPEP, and FMPEP-$d_2$) were measured specific binding of $^{11}$C-SD5024 in the monkey brain, and compared this with the specific binding of $^{11}$C-MePPEP and $^{18}$F-FMPEP-$d_2$ (Jenko et al., 2012; Zoghbi et al., 2012) (Fig. 1). In healthy humans, we measured the brain uptake and washout of $^{11}$C-SD5024, calculated $V_t$ and its intersubject variability as an indirect measure of the precision of the quantification, and compared these with the published results of the other four ligands.

To image high density target such as CB$_1$ receptor using $^{11}$C-labeled PET binding and smaller intersubject variability are desired. Appropriate lipophilicity for brain imaging is LogD$_{2,4}$ between 2 and 4 (Waterhouse, 2003). In brain scans, higher levels of specific binding and smaller intersubject variability are desired.

**Material and methods**

**In vitro experiments**

**Binding assay**

In vitro receptor binding assays were performed as previously described with minor modifications (Jenko et al., 2012). Briefly, the human parietal cortex was homogenized in buffer (20 mM HEPES, 5 mM MgCl$_2$, 1 mM EDTA, pH 7.4) with a Teflon pestle using a Glass-Col Homogenizing System and centrifuged at 25,000 $\times g$ for 25 min at 4 °C. The pellet was re-suspended, aliquoted, and stored at $-80$ °C. Protein concentration was determined using the Bradford Protein Assay (Bio-Rad, Hercules, CA).

To determine the affinity ($K_i$ and $IC_{50}$) of SD5024, MePPEP (Merck Research Laboratories, West Point, PA) for the CB$_1$ receptor, a heterologous binding assay was performed on one brain sample, in triplicate in each of two separate assays for a total of 6.100 $\mu$L of $[3H]$MePPEP (specific activity 3.07 GBq/mol; ~0.11 nM, diluted in buffer with 0.5% w/v BSA; Amersham GE Healthcare, UK) was added to each assay tube, followed by 100 $\mu$L of 12 concentrations (0.001 nM–3 μM) of the displacing ligand, 100 $\mu$L buffer (to determine total binding), or 1 μM rimonabant (Eli Lilly, Indianapolis, IN) (to determine nonspecific binding). 800 $\mu$L of human parietal cortex suspension (41 μg/mL protein) was added and incubated for 90 min in a shaking water bath at 23 °C. Samples were filtered with a Brandel cell harvester (Gaithersburg, MD) through a Whatman GF/A filter paper, followed by three washes of 3 mL ice-cold 50 mM Tris–HCl buffer (pH = 7.4; 4 °C).

![Chemical structures of five PET ligands for CB$_1$ receptor.](https://example.com/structures.png)

**Fig. 1.** Chemical structures of five PET ligands for CB$_1$ receptor.
Radioactivity was measured with liquid scintillation counting for 5 min using 4 mL of Ultima-Gold (Perkin Elmer, Chicago, IL).

Data were analyzed for \( K_r \) and \( K_{SO} \) using nonlinear regression curve-fitting software provided by GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The \( K_r \) for MePPEP, 0.31 nM (Jenko et al., 2012), was used in the determination of \( K_r \).

Lipophilicity

The value of \( \log D_{7,4} \) was measured at room temperature as previously described (Briard et al., 2008; Zoghbi et al., 1997, 2012). In brief, approximately 22–170 MBq of \(^{11}\text{C}-\text{MePPEP} \) and 26 MBq of \(^{18}\text{F}-\text{FMPEP-d}_2 \) (radiochemical purities > 99.6%) were separately added to each of six tubes in 1.0 mL of 0.15 M sodium phosphate buffer (pH 7.4). To each of the test tubes, a 1.0 mL of n-octanol was added and the contents of each test tube were vortexed for 1.0 min. The tubes were then centrifuged at 1800 g for 1.0 min after which the two phases were separated and aliquots (200 \( \mu \)L, each) from each phase were counted in an automatic \( \gamma \)-counter. The counts of the aqueous phase were then corrected using the results of the radio-HPLC analysis of the aqueous phases. The average measured \( \log D_{7,4} \) was then calculated according to the formula:

\[
\log D_{7,4} = \log\left(\frac{\text{cpm organic phase}}{\text{corrected cpm aqueous phase}}\right)_{7,4}.
\]

Samples that contained high levels of radioactivity, outside the optimal sensitivity range of the counter, were allowed to decay until the dead time factor of the counter became normal. Samples with low counts, usually the aqueous phases, were counted first and their measured counting errors (SD/mean) were 4.6% ± 0.3%, 1.8% ± 0.2%, and 0.8% ± 0.07% (n = 6 for each) for \(^{11}\text{C}-\text{MePPEP} \), \(^{18}\text{F}-\text{FMPEP-d}_2 \), and \(^{11}\text{C}-\text{SD5024} \), respectively.

**Monkey PET**

**Radioligand preparation**

\(^{11}\text{C}-\text{SD5024} \) was prepared as described above. The radiochemical purity was 100%, and the specific activity was 21 ± 10 GBq/\( \mu \)mol at times of injection (n = 7 batches).

**Human PET**

Approval for this study was obtained from the Combined Neurosciences Institutional Review Board of the National Institute of Mental Health and the Radiation Safety Committee of the National Institutes of Health. Seven healthy volunteers participated in the brain PET scans (3 males, 4 females; 30 ± 6 years of age). All subjects were free of current medical or psychiatric illnesses.

**Measurement of \(^{11}\text{C}-\text{SD5024} \) in the plasma**

To determine arterial input function for brain PET scans, blood samples (1 mL each) were drawn from the femoral artery at 15-second intervals until 150 s, followed by 3 mL samples at 3, 4, 6, 8, 10, 15, 20, 30, 40, and 50 min, and 5 mL at 60, 75, 90, and 120 min. The concentration of parent radioligand was measured using HPLC as described above for monkey studies.

**Scan procedures**

All PET scans were performed on an Advance tomograph (GE Medical Systems, Waukesha, WI). \(^{11}\text{C}-\text{SD5024} \) (418 ± 177 MBq) was intravenously injected over 1 min, and dynamic three-dimensional emission scans were acquired for 120 min in 33 frames. Head movement was corrected after the scan by realigning all frames from each subject using Statistical Parametric Mapping, SPM (Version 8 for Windows, Wellcome Department of Cognitive Neurology, UK). The position of the transmission scan was corrected for motion before applying attenuation correction. PET images were reconstructed with filtered back projection.

**Data analysis**

For monkey scans, the time-activity curves of \(^{11}\text{C}-\text{SD5024} \) concentrations in arterial plasma were fitted to a tri-exponential function.

![Fig. 2. Displacement curves of \(^{3}\text{H}\)-MePPEP by five \( \text{CB}_1 \) ligands in human parietal cortex homogenate. SD5024 (\( \square \)), FMPEP-d_2 (\( \circ \)), MePPEP (\( \triangle \)), MK-9470 (\( \gamma \)), OMAR (\( \Delta \)). Data represent mean ± 95% confidence interval in nM (n = 6).](https://www.nature.com/articles/ncomms2345/figs/fig2.pdf)
In vitro Kᵣ of CB₁ receptor ligands measured in the human parietal cortex.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Kᵣ (nM)</th>
<th>95% CI (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-9470</td>
<td>0.10</td>
<td>0.09–0.11</td>
</tr>
<tr>
<td>MePPEP</td>
<td>0.11</td>
<td>0.10–0.12</td>
</tr>
<tr>
<td>FMPEP-d₂</td>
<td>0.11</td>
<td>0.10–0.13</td>
</tr>
<tr>
<td>SD5024</td>
<td>0.47</td>
<td>0.42–0.54</td>
</tr>
<tr>
<td>OMAR</td>
<td>2.05</td>
<td>1.82–2.32</td>
</tr>
</tbody>
</table>

Data represent mean and 95% confidence interval (CI) in nM (n = 6).

**Image processing**

For both monkey and human PET, regional radioactivity was obtained by using a set of preset volumes of interest and MRI coregistered to PET (Tzourio-Mazoyer et al., 2002; Yasuno et al., 2002). Regional data for the following 10 regions were obtained: frontal, parietal, occipital, temporal, and medial temporal cortices; caudate; putamen; thalamus; cerebellum; and white matter. Realignment, coregistration, and spatial normalization were performed using SPM8. The regional and kinetic analyses were performed using pixelwise modeling software (PMOD 3.16, PMOD Technologies Ltd., http://www.pmod.com/).

**Compartmental modeling.** Brain time-activity data were analyzed with both one- and unconstrained two-tissue compartment models. Rate constants (kᵣ, k₂, k₃, and k₄) and percentage of vascular compartment in tissue volume (Vb) in standard one- and two-tissue compartment models were estimated with the weighted least-squares method and the Marquardt optimizer. Brain data for each frame were weighted by assuming that the standard deviation/mean of the data was proportional to the inverse square root of noise equivalent counts. The delay between the arrival of ¹¹C-SD5024 in the radial artery and brain was estimated by fitting the whole brain, excluding the white matter.

Because in vitro studies showed that no brain region lacks CB₁ receptor expression (Glass et al., 1997; Herkenham et al., 1990), we did not apply a reference region method in the kinetic analysis.

**Time stability**

In human studies, to determine the minimal scan length for reliable measurements and also to indirectly assess whether ¹¹C-SD5024 radiotracers enter the brain, time stability of Vᵣ was examined by increasingly truncating the 120-min scan by 10-minute increments to the shortest length of 0 to 40 min.

**Statistical analysis**

The optimal compartment model (i.e., one- vs. two-tissue compartment) was chosen based on the Akaike information criterion (AIC).
model selection criterion (MSC proposed by Micromath®, Saint Louis, MO, http://www.micromath.com/products.php?p=statistical_analysis), and F-test. The more appropriate model is the one with the smaller AIC and the larger MSC value. F-statistics were used to compare goodness-of-fit by one- and two-tissue compartment models. A value of \( p < 0.05 \) was considered significant. The identifiability (%) of vascular component and rate constants was expressed as a percentage and equaled the ratio of the standard error (SE) of the kinetic variables divided by the value of the kinetic variables themselves. Identifiability (%) of \( V_T \) was calculated from the covariance matrix using the generalised form of error propagation equation (Bevington and Robinson, 2003), where correlations among parameters \( (K_1 \) and \( k_2 \) or \( k_3, k_4, \) and \( k_5) \) were taken into account. A lower percentage indicates better identifiability.

All statistical analyses were performed using SPSS (Version 17 for Windows, SPSS Inc, Chicago, IL). Group data are expressed as mean ± SD.

## Results

### In vitro experiments

#### Binding assay

All five CB₁ receptor ligands showed the presence of one, but not two, binding sites in the inhibition curves of \(^{11}C\)-MePPEP (Fig. 2). Three ligands (FMPEP-d₂, MePPEP, and MK-9470) had high affinity of 0.10 to 0.11 nM (Table 1). OMAR had comparatively low affinity (2 nM), and SD5024 had intermediate affinity (0.47 nM).

#### Lipophilicity

The measured lipophilicity index, \( \text{LogD}_{7.4} \) of \(^{11}C\)-SD5024 (3.79 ± 0.09) was markedly lower than that of \(^{11}C\)-MePPEP (4.77 ± 0.27) and moderately lower than that of \(^{18}F\)-FMPEP-d₂ (4.24 ± 0.08; 6 measurements for each ligand). That is, a difference of one log unit (3.8 ± 4.8) reflects a ten-fold difference in lipophilicity.

#### Monkey PET

### Brain radioactivity and kinetic analysis

Following injection of \(^{11}C\)-SD5024, brain activity increased to moderate levels (~2 SUV) at ~60 min followed by slow washout (Fig. 3). The distribution of activity was consistent with binding to CB₁ receptors, with high levels in the striatum and low levels in the thalamus. The brain uptake of \(^{11}C\)-SD5024 was markedly reduced with receptor saturating dose of rimonabant (3 mg/kg i.v.) (Fig. 3).

For the kinetic analysis, one-tissue compartmental fitting of time-activity curves converged in all regions and in all scans, but unconstrained two-tissue compartmental fitting did not converge in 11 of 64 fittings in 4 scans. An F-test showed that the two-compartment model did not significantly improve goodness-of-fit compared to one-compartment model in 42 of 53 fittings where the two-compartment model converged. The one-compartment model well identified \( V_T \) with average SE across brain regions of 3.8%. Regional \( V_T \) values (mL·cm⁻³) were measured from two monkeys under baseline condition were consistent with the regional rank order of \( V_T \) values in the monkey brain previously reported by using \(^{11}C\)-MePPEP (Yasuno et al., 2008), showing high level in the putamen, medium in the lateral temporal cortex, and low in the thalamus (Table 2).

### Human PET

#### Pharmacological effects

The injected mass dose of \(^{11}C\)-SD5024 was 242 ± 47 pmol/kg (\( n = 7 \)), which caused no pharmacological effects, based on subjective reports, vital signs, and laboratory tests.

#### Plasma analysis

\(^{11}C\)-SD5024 concentrations in arterial plasma peaked to 24 ± 8 SUV at 75 s after \(^{11}C\)-SD5024 injection, and then rapidly declined to a slow terminal clearance phase (Fig. 4A). The fraction of \(^{11}C\)-SD5024, expressed as a percentage of total plasma radioactivity, declined slowly and remained 51 ± 13% even at 75 min (Fig. 4B). The fitting of whole blood and total plasma curves converged by tri-exponential function (not shown), and that of parent fraction curve converged by a Hill function in all subjects. Multiplication of fraction of parent (Fig. 4B) and total plasma activity provided \(^{11}C\)-SD5024 concentrations in arterial plasma (Fig. 4A).

Radiometabolites appeared slowly in the plasma and became the predominant component of plasma radioactivity after 90 min. All radiometabolites eluted before the more lipophilic parent by reverse-phase HPLC (Fig. 4C). The parent radioligand eluted at 3.5 min and was well separated from the radiometabolites.

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Brain radioactivity and kinetic analysis

After 11C-SD5024 injection, activity peaked at a moderate concentration (SUV = 1.5–3) at ~40 min, followed by slow washout in all brain regions (Figs. 5, 6). That is, brain radioactivity decreased by only 20% from peak time (40 min) to the end of the scan (120 min). The brain time-activity curves showed a transient and early peak at about 1 min after 11C-SD5024 injection, activity peaked at a moderate concentration (SUV = 1.5–3) at ~40 min, followed by slow washout in all brain regions (Figs. 5, 6). That is, brain radioactivity decreased by only 20% from peak time (40 min) to the end of the scan (120 min). The brain time-activity curves showed a transient and early peak at about 1 min after 11C-SD5024 injection. After subtracting activity of vasculature from that of brain regions, this early peak disappeared (Fig. 6B). Thus, this early peak reflected transiently high concentrations of radioactivity in the blood.

Kinetic analysis of brain and plasma data had three major results: 1) Brain uptake was better fit by one- than two-tissue-compartment model, consistent with the majority of brain uptake being specific binding as found in monkey (Table 2). 2) Only the initial 80 min of scan data was adequate to stably measure $V_T$. 3) The intersubject variability of $V_T$ was low, suggesting that 11C-SD5024 provided relatively precise measurements.

First, one-compartmental fitting converged in all regions and in all scans, but unconstrained two-compartmental fitting did not converge in 31 of 70 fittings in 7 scans. This result is consistent with the majority of brain uptake having one kinetic profile — i.e., the predominant uptake being in the specific (i.e., receptor-bound) compartment. In addition, in 39 fittings where both one- and two-compartment models converged, the former showed better goodness-of-fit than the latter, based on AIC and MSC scores and the F-test. One-compartment model showed lower mean AIC scores (252 vs. 259) and higher mean MSC scores (3.6 vs 3.5) than the unconstrained two-compartment model. An F-test showed that the two-compartment model did not significantly improve goodness-of-fit than one-compartment model. The one-compartment model well identified $V_T$ with average SE across brain regions of 1.8%. Regional $V_T$ values were consistent with known distribution of CB1 receptors, showing high level in the putamen, medium in the frontal cortex, and low in the thalamus (Table 3).

Second, despite the moderately slow washout of the radioligand from the brain, only the initial 80 min of scanning provided values of $V_T$ equivalent to that using the complete 120 min of scanning. To determine the minimal scanning time to give accurate $V_T$, we increasingly truncated the entire scan by 10-min increments from 0–120 to 0–40 min but displayed only half of these intervals (Fig. 7). As expected, short scanning lengths (e.g., 40 and 60 min) overestimated $V_T$, especially in highest density regions, which are the latest to achieve peak uptake.

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Scanning for 80 or 100 min provided values which were essentially equivalent to those from 120 min, which is regarded in this analysis as the “correct” value. These results not only show that 80 min of scanning with 11C-SD5024 is adequate to measure CB₁ receptors but also suggest that radiometabolites do not significantly accumulate in the brain. That is, brain uptake was fully and stably determined by 80 min from the input function of only the parent radioligand in the plasma.

As background to the third result, we previously found that 11C-MePPEP had high intersubject variability (~60%), which was caused in large part by noise (imprecision) in the measurement of low concentrations of radioligand in the plasma, especially at late time points (Terry et al., 2009). As an indirect measure of precision of 11C-SD5024, the intersubject variability of Vₜ (as an indirect measure of precision) (Tables 5, 6). For the 11C-labeled radioligands, the peak brain uptake of 11C-SD5024 was about 20% lower than that of 11C-MePPEP but about 40% higher than that of 11C-OMAR (Table 5). Based on only brain uptake, 11C-SD5024 appears superior to 11C-OMAR, but the actual impact on quantitation of 11C-OMAR also depends on reproducibility of both plasma and brain measurements. 11C-SD5024 showed about twice higher V₀ values than 11C-OMAR, but Vₜ of 11C-SD5024 was only 15–20% of that for 18F-MK-9470, 11C-MePPEP, and 18F-FMPEP-d₂ (Table 6). Greater V₀ values may reflect higher levels of in vivo affinity. However, the presence of two unknown parameters should be noted: nondisplaceable distribution volume and the free fraction in the plasma (f₀) (see Discussion (Innis et al., 2007).

11C-SD5024 showed low intersubject variability (~24%) for Vₜ (Table 6), indicating good precision. The intersubject variability of 11C-SD5024, 11C-OMAR, and 18F-FMPEP-d₂ was markedly smaller than that of 11C-MePPEP and 18F-MK-9470, indicating good precision. The intersubject variability of 11C-MePPEP and 18F-MK-9470 was significantly lower than that of 11C-OMAR and 18F-FMPEP-d₂ (Table 6).

**Comparison on in vivo binding of 11C-SD5024 with that of the other PET ligands**

The specific binding of 11C-SD5024 in the monkey brain (measured at baseline and after receptor blockade) was high and represented 71–82% of Vₜ (mean of two monkey studies, Table 2). Specific binding of 11C-SD5024 was similar to that of 11C-MePPEP and 18F-FMPEP-d₂ (Table 4).

For human studies, we compared 11C-SD5024 and the four other CB₁ radioligands with regard to peak brain uptake (which reflects the magnitude of the brain signal per unit of injected activity), Vₜ and the intersubject variability of Vₜ (as an indirect measure of precision) (Tables 5, 6). For the 11C-labeled radioligands, the peak brain uptake of 11C-SD5024 was about 20% lower than that of 11C-MePPEP but about 40% higher than that of 11C-OMAR (Table 5). Based on only brain uptake, 11C-SD5024 appears superior to 11C-OMAR, but the actual impact on quantitation of 11C-OMAR also depends on reproducibility of both plasma and brain measurements. 11C-SD5024 showed about twice higher V₀ values than 11C-OMAR, but Vₜ of 11C-SD5024 was only 15–20% of that for 18F-MK-9470, 11C-MePPEP, and 18F-FMPEP-d₂ (Table 6). Greater V₀ values may reflect higher levels of in vivo affinity. However, the presence of two unknown parameters should be noted: nondisplaceable distribution volume and the free fraction in the plasma (f₀) (see Discussion (Innis et al., 2007).

11C-SD5024 showed low intersubject variability (~24%) for Vₜ (Table 6), indicating good precision. The intersubject variability of 11C-SD5024, 11C-OMAR, and 18F-FMPEP-d₂ was markedly smaller than that of 11C-MePPEP.

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**Table 3**

Parameters measured with one-tissue compartment model in the human brain.

<table>
<thead>
<tr>
<th>Region</th>
<th>Vₜ (%)</th>
<th>Standard error (%)</th>
<th>Kᵣ (mL·cm⁻³·min⁻¹)</th>
<th>Standard error (%)</th>
<th>k₂ (min⁻¹)</th>
<th>Standard error (%)</th>
<th>V₀ (mL·cm⁻³)</th>
<th>Standard error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>4.4 ± 0.66</td>
<td>4.67</td>
<td>0.032 ± 0.006</td>
<td>0.72</td>
<td>0.013 ± 0.003</td>
<td>2.02</td>
<td>2.57 ± 0.54</td>
<td>1.43</td>
</tr>
<tr>
<td>Parietal</td>
<td>4.8 ± 0.50</td>
<td>4.73</td>
<td>0.034 ± 0.006</td>
<td>0.80</td>
<td>0.014 ± 0.003</td>
<td>2.11</td>
<td>2.44 ± 0.50</td>
<td>1.45</td>
</tr>
<tr>
<td>Occipital</td>
<td>5.9 ± 0.49</td>
<td>3.94</td>
<td>0.035 ± 0.007</td>
<td>0.87</td>
<td>0.018 ± 0.003</td>
<td>1.96</td>
<td>2.00 ± 0.38</td>
<td>1.26</td>
</tr>
<tr>
<td>Lateral temporal</td>
<td>5.2 ± 0.64</td>
<td>4.12</td>
<td>0.032 ± 0.007</td>
<td>0.77</td>
<td>0.012 ± 0.002</td>
<td>2.25</td>
<td>2.66 ± 0.57</td>
<td>1.61</td>
</tr>
<tr>
<td>Medial temporal</td>
<td>5.3 ± 0.58</td>
<td>4.22</td>
<td>0.021 ± 0.005</td>
<td>1.05</td>
<td>0.010 ± 0.002</td>
<td>3.58</td>
<td>2.48 ± 0.65</td>
<td>2.69</td>
</tr>
<tr>
<td>Cingulate</td>
<td>5.0 ± 0.58</td>
<td>4.94</td>
<td>0.029 ± 0.006</td>
<td>0.94</td>
<td>0.011 ± 0.002</td>
<td>3.05</td>
<td>2.79 ± 0.58</td>
<td>2.27</td>
</tr>
<tr>
<td>Caudate</td>
<td>3.7 ± 0.67</td>
<td>7.26</td>
<td>0.028 ± 0.005</td>
<td>1.07</td>
<td>0.013 ± 0.003</td>
<td>3.01</td>
<td>2.31 ± 0.55</td>
<td>2.14</td>
</tr>
<tr>
<td>Putamen</td>
<td>4.0 ± 0.79</td>
<td>8.21</td>
<td>0.038 ± 0.008</td>
<td>0.94</td>
<td>0.013 ± 0.003</td>
<td>2.83</td>
<td>1.71 ± 0.41</td>
<td>1.71</td>
</tr>
<tr>
<td>Thalamus</td>
<td>4.6 ± 0.49</td>
<td>5.53</td>
<td>0.029 ± 0.006</td>
<td>1.17</td>
<td>0.017 ± 0.004</td>
<td>2.66</td>
<td>1.71 ± 0.41</td>
<td>1.71</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>6.4 ± 0.69</td>
<td>3.73</td>
<td>0.030 ± 0.006</td>
<td>1.03</td>
<td>0.016 ± 0.003</td>
<td>2.45</td>
<td>1.93 ± 0.38</td>
<td>1.61</td>
</tr>
</tbody>
</table>

---

**Fig. 7.** Effect of increasing scan duration on total distribution volume (Vₜ) determined with a one-tissue compartment model. Correlation between Vₜ calculated from the complete 120 min of scanning (x-axis) and that calculated from the initial (A) 40, (B) 60, (C) 80, or (D) 100 min of scanning (y-axis). The dots represent 70 regions from 7 subjects. The dotted line in each graph is the line of identity.
The purpose of that study was to determine the causes of the large intersubject variability of $^{11}$C-MePPEP compared to $^{18}$F-FMPEP-$d_2$ when we did not know the actual variation in receptor density in the study population. Because distribution volume ($V_T$) is a ratio of concentration of radioactivity in the brain to that in the plasma, the restest paradigm allowed identification with moderate certainty of the relative contributions of measurement errors (noise) in the calculation of $V_T$. That study somewhat surprisingly showed that the errors in measuring the concentrations of radioligand in the plasma were more problematic to calculate $V_T$ than measuring radioligand in the brain. That is, the large intersubject variability of $V_T$ using $^{11}$C-MePPEP was largely caused by the poor precision in the measurement of low concentrations of radioligand in the plasma, particularly at late time points (Terry et al., 2009, 2010). The current study of $^{11}$C-SD5024 may have achieved a more accurate arterial input function than that of $^{11}$C-MePPEP because the plasma concentrations of $^{11}$C-SD5024 were much higher than those of $^{11}$C-MePPEP. For example, the plasma concentrations from 30 to 120 min of $^{11}$C-SD5024 were five-times that of $^{11}$C-MePPEP (Fig. 4A and Terry et al., 2009).

Our prior comparison of $^{11}$C-MePPEP and $^{18}$F-FMPEP-$d_2$ showed the utility of a restest paradigm to identify contributors to the intersubject variability of $V_T$ from noisy (imprecise) measurements in the brain and plasma. In the absence of a restest paradigm (as in the current study), intersubject variability by itself is merely an indirect measurement of precision. For example, the large intersubject variability reported for one radioligand may reflect the actual biological variability in a diverse study population. Nevertheless, our experience with comparison of $^{11}$C-MePPEP and $^{18}$F-FMPEP-$d_2$ showed that large intersubject variability of $V_T$ suggests, but does not prove, relatively noisy measurements in either the brain or plasma.

Specific binding in human, i.e., $V_d/f_0$, has not been measured for any of the five CB$_1$ ligands. Nevertheless, based on currently available data of indirect measures of specific binding, i.e., $V_d$ and peak brain uptake in human, $^{11}$C-SD5024 appears to be slightly better than $^{11}$C-MePPEP. To determine which $^{11}$C-labeled ligand is the best, particularly for the comparison between $^{11}$C-SD5024 and $^{11}$C-MePPEP, receptor occupancy studies in human are required where specific binding is measured by comparing $V_d/f_0$ under baseline and receptor blockade. Because $f_0$ is too low to measure accurately for $^{11}$C-SD5024 or not reported for $^{11}$C-MePPEP, currently, $V_d$ and peak brain uptake in human (Table 5, 6) are the most useful parameters to compare $^{11}$C-SD5024 and $^{11}$C-MePPEP. Although peak uptake is a crude parameter, it may reflect how much free ligand enters the brain and binds to the receptor. $^{11}$C-SD5024 showed ~1.4 times greater peak brain uptake (Table 5) and about twice greater $V_d$ (Table 6) than $^{11}$C-MePPEP. These human PET data are in line with the results of the in vitro experiments where SD5024 showed four times greater affinity than OMeP (Table 1). However, a comparison based on $V_d/f_0$ is still needed.

**Conclusions**

$^{11}$C-SD5024 showed appropriate lipophilicity, high specific binding in the monkey brain, and good precision for measuring CB$_1$ receptors in humans. $^{11}$C-SD5024 is, therefore, a promising ligand to image CB$_1$ receptors.
Table 6

Comparison of VT and intersubject variability (SD/mean) among five CB1 receptor ligands.

<table>
<thead>
<tr>
<th>11C-labeled ligands</th>
<th>11C-OMAR</th>
<th>11C-MePPEP</th>
<th>11C-SD5024</th>
<th>18F-FMPEP</th>
<th>18F-MK-9470</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region (n = 10, 90 min)</td>
<td>Region (n = 17, 150 min)</td>
<td>Region (n = 7, 120 min)</td>
<td>Region (n = 9, 120 min)</td>
<td>Region (n = 12, 700, 360, 150 min)</td>
<td></td>
</tr>
<tr>
<td>Intersubject</td>
<td>Intersubject</td>
<td>Intersubject</td>
<td>Intersubject</td>
<td>Intersubject</td>
<td></td>
</tr>
<tr>
<td>VT (ml·cm⁻³)</td>
<td>Variability</td>
<td>VT (ml·cm⁻³)</td>
<td>Variability</td>
<td>VT (ml·cm⁻³)</td>
<td>Variability</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>1.47 ± 0.42</td>
<td>17%</td>
<td>Frontal</td>
<td>22.4 ± 12.8</td>
<td>57%</td>
</tr>
<tr>
<td>Cingulate</td>
<td>1.23 ± 0.16</td>
<td>21%</td>
<td>Thalamus</td>
<td>11.2 ± 5.0</td>
<td>44%</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.32 ± 0.20</td>
<td>15%</td>
<td>Putamen</td>
<td>29.1 ± 17.4</td>
<td>60%</td>
</tr>
<tr>
<td>11C-SD5024</td>
<td>2.57 ± 0.54</td>
<td>21%</td>
<td></td>
<td>22.7 ± 6.4</td>
<td>28%</td>
</tr>
<tr>
<td>18F-FMPEP</td>
<td>1.71 ± 0.41</td>
<td>24%</td>
<td></td>
<td>9.6 ± 2.5</td>
<td>26%</td>
</tr>
<tr>
<td>18F-MK-9470</td>
<td>3.15 ± 0.70</td>
<td>22%</td>
<td></td>
<td>24.3 ± 7.2</td>
<td>28%</td>
</tr>
</tbody>
</table>

Values of VT are shown as mean ± SD and intersubject variability is calculated by SD / mean.

References

This study was supported by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH) and by the 2011/2013 Wagner-Torizuka Fellowship of Society of Nuclear Medicine. We thank Maria Ferraris Araneta, Denise Rallis-Frutos, Gerald Hodges, David Clark, Jeih-San Liow, and the staff of the PET Department for assistance in successful completion of the studies, and PMOD Technologies (Zurich, Switzerland) for providing its image analysis and modeling software. We thank Andrew Horti (Johns Hopkins) for providing a sample of OMAR and Terence Hamill (Merck Research Laboratories) for providing a sample of MK-9470.

Conflict of interest statement

There are no conflicts of interest.

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